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A new species of *Pisolithus* from Spain

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Abstract — *Pisolithus calongei* is described as a new species based on our morphological observations and comparison of ITS nrDNA sequences.

Key words — *Basidiomycota*, *Cistus*, *Boletales*, taxonomy, *Pisolithus tinctorius*

Introduction

During their overview of the donkey dung fungus (*Pisolithus* Alb. & Schwein.) from many regions of the world using molecular and classical methods, Phosri et al. (2012) analysed collections from Europe. All generated molecular trees grouped Spanish specimens into three clades, of which two represent the classic *Pisolithus tinctorius* (Pers.) Coker & Couch and *P. arhizus* (Scop.) Rauschert. However, four sequences obtained from *Pisolithus* specimens associated with *Cistus ladanifer* L. (*Cistaceae*) formed a separate group more closely related to *P. marmoratus* (Berk.) E. Fisch. (occurring in *Eucalyptus* spp. plantations from Australia, China, and Kenya) than to other Spanish specimens.

Cistaceae is well documented as an ectomycorrhizal phanerogam family (Harley & Harley 1987), although many species also form VA and ectendomycorrhizas. Association-forming species are widespread amongst the larger fungi, e.g. *Leccinum arenicola* Watling & Redhead, found in North America with *Hudsonia* L. (Redhead & Watling 1979), and *Terfezia alsheikhii* Kovács et al., found in Spain with *Helianthemum* Mill. (Kovács et al. 2011). In Europe a number of fungi associate with *Helianthemum*, including *Boletus* spp. (Águeda et al. 2006, 2008; Ponce et al. 2011), *Laccaria laccata* (Scop.) Cooke (Torres et al. 1995), and *Cortinarius* spp. and *Hebeloma* spp. (Watling 1988; Moreau 2002; Henrici 2003, 2005). Even *Astraeus hygrometricus* (Pers.)

Morgan, a close relative of *Pisolithus,* has been associated with *Cistus* in Spain (Martín 1988, specimens in BCN; M. Jeppson personal collection with duplicate specimens in E).

Recently, three *Pisolithus* specimens were collected near *Cistus ladanifer* in Spain. Phylogenetic analyses performed according to Phosri et al. (2012) separated the specimens into two distinctive taxa (Fig. 1): one collection (MA-Fungi 47734 (duplicates at BH & E): J.C. Campos & E. Ramírez MPM 3320 collectors near El Robledillo, 435-465 m alt. on 4-Dec-2010; GenBank: HE578142) grouped in the *'tinctorius'* clade and the other two collections (MA-Fungi 47732, MA-Fungi 47733) grouped in the "*Pisolithus* species 3" subclade from Spain together with sequences AF228641–AF228644 (Díez et al. 2001) from specimens from Alcalá de Henares Univ. Herbarium (AH) and FR748140/ FR748142 (Phosri et al. 2012) from the MJ6192 collection (M. Jeppson personal herbarium) revised here.

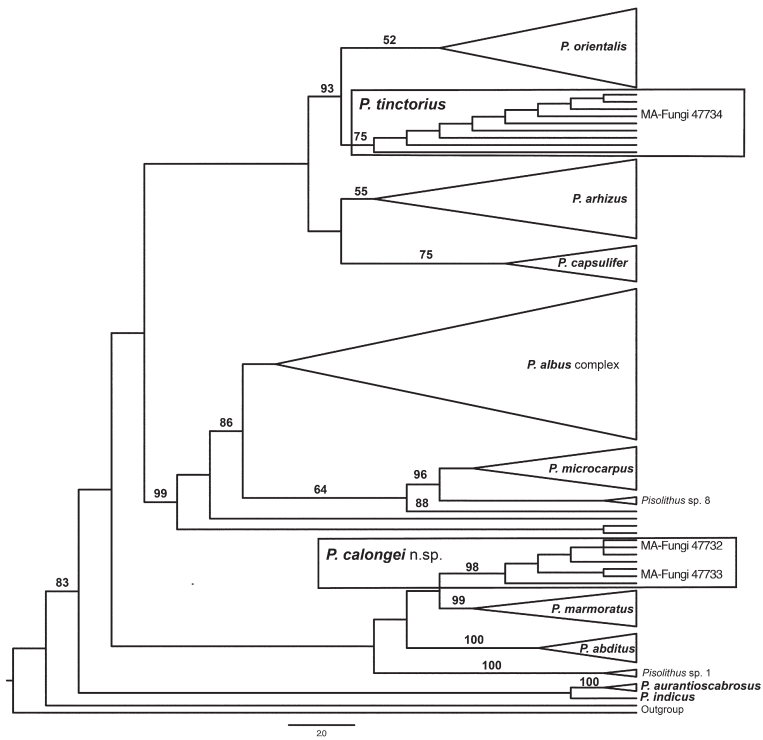

Fig. 1. *Pisolithus calongei* and other *Pisolithus* spp. One of the 100 most parsimony trees obtained under heuristic search (PAUP* 4.0b10) using the sequences included in Phosri et al. (2012) and three newly generated sequences from our *Pisolithus* collections from Spain. The parsimony bootstrap values (%) are indicated on the branches. Bar = 20 changes.

Identification of microscopic features distinctive to MA-Fungi 47732 and MA-Fungi 47733 associated with *C. ladanifer* further support our belief that the specimens represent a new species, which we describe here.

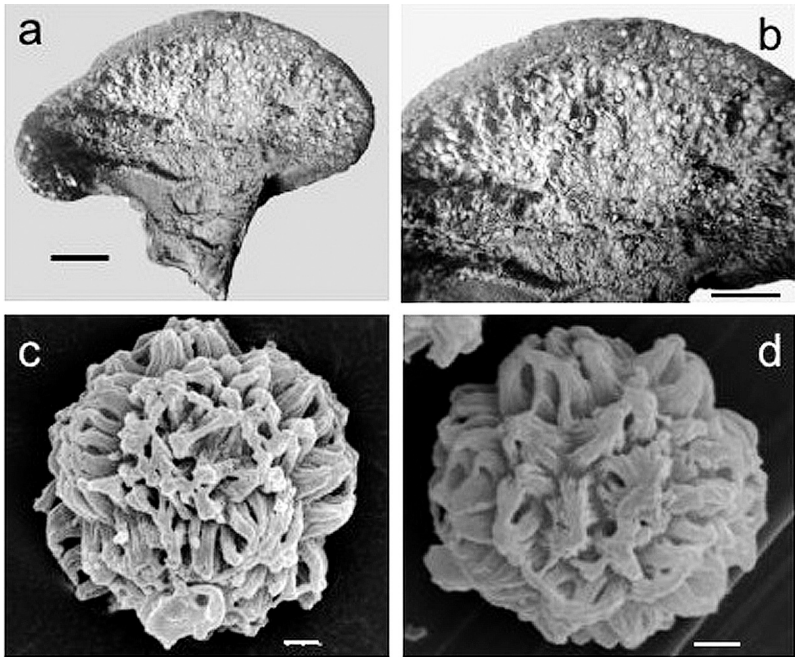

Fig. 2. *Pisolithus calongei.* a–b (MA-Fungi 47732): Turbinate gastrocarp. c–d (Holotype, MA-Fungi 47733): Basidiospores (SEM) coarsely ornamented by broad connate structures that form irregular pyramid-like spines. Scale bars: a = 1 cm; b = 0.5 cm; c–d = 1 μm.

Taxonomy

Pisolithus calongei M.P. Martín, Phosri & Watling, **sp. nov.** Figs 2–3

MycoBank MB 563437

Differs from *Pisolithus tinctorius* and *P. arhizus* by its turbinate habit and from all *Pisolithus* spp. in its ITS nrDNA sequence.

Type: Spain. Ávila: Candeleda, Las Atalayas, 30TU-00477, 355 m alt., close to *Cistus ladanifer*, 11-Dec-2010, leg. E. Ramírez & J. C. Campos MPM 3319 (**Holotype**, MA-Fungi 47733; **isotypes**, BH, E; GenBank, HE578141).

Etymology: Honouring Francisco D. Calonge, who has worked so diligently on the Iberian gasteromycete flora.

Gastrocarp turbinate, pulvinate, 32–40 mm broad, 28–45 mm high, with shortened stipe 6–14 × 10–13 mm, with abrupt base, ± attached to soil mass, main primary peridial layer 15–24 mm deep. Peridium irregular, at most 0.5

Fig. 3. *Pisolithus calongei* (M. Jeppson MJ6192). Turbinate gastrocarp.

mm thick, but soon thinning to show outline of peridioles below, even stretching to accommodate complete peridioles, finally disintegrating and leaving small islands of original ferruginous peridium above yellow-ochre peridiole surfaces or bruising to form lacquer-like blackened smudges. Peridioles 1–1.5 mm diam. (rarely <2 mm), ochraceous yellow at first, later ferruginous brown, compressed in uppermost part, often forming in stipe apex or even developing within the entire stipe to produce irregular rugulose outer surface on the stipe, concolorous with and non-differentiated from the peridial surface; upper peridium finally eroding away to expose the disintegrating peridioles as a dry ferruginous powdery mass. Basidiospores globose 8.6–11 µm diam., coarsely ornamented with broadly connate structures that form irregular pyramid-like spines, 1.5 µm high, rarely collapsing to give broader structures.

Additional specimens examined: **SPAIN. Ávila**: Candeleda, Las Atalayas, 30TU-00477, 350 m alt., close to *Cistus ladanifer*, 4-Dec-2010, leg. E. Ramírez & J. C. Campos MPM3318 (MA-Fungi 47732; duplicate, BH; GenBank, HE578140). **Huelva**: Las Palmas, 1-Jan-2003, leg. M. Jeppson MJ6192 (M. Jeppson personal herbarium).

Pisolithus calongei is distinctive in the field in its turbinate habit, close fruiting to the soil-surface and therefore being concealed by the *Cistus* with which it is thought to associate. The collectors observed no *Pinus* species in the vicinity. The new species may have been overlooked in the past or identified as *P. arhizus* or *P. tinctorius*. Nonetheless, the ITS nrDNA sequence analysis (Fig. 1) clearly separates *P. calongei* from the other three European species

(*P. arhizus*, *P. capsulifer* (Sowerby) Watling et al., and *P. tinctorius*), as well as from the other species previously analyzed: *P. albus* complex, *P. abditus* Kanch. et al., *P. aurantioscabrosus* Watling, *P. indicus* Natarajan & Senthil., *P. marmoratus* (Berk.) E. Fisch., *P. microcarpus* (Cooke & Massee) G. Cunn., and *P. orientalis* Watling et al. (Watling et al. 1995, Martin et al. 2002, Kanchanaprayudh et al. 2003a,b, Moyersoen et al. 2003, Reddy et al. 2005, Phosri et al. 2012).

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